MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

DATE: May 5, 1997

FROM: Director, Center for Drug Evaluation and Research

SUBJECT: Approvability of a Synthetic Generic Version of Premarin

TO: Douglas L. Sporn
Director, Office of Generic Drugs

I. Introduction

This memorandum transmits the Center for Drug Evaluation and Research's (CDER) position on the circumstances under which an abbreviated new drug application (ANDA) for a synthetic version of Premarin could be approved at this time. The Center's conclusion is that because the reference listed drug Premarin is not adequately characterized at this time, the active ingredients of Premarin cannot now be definitively identified. Until the active ingredients are sufficiently defined, a synthetic generic version of Premarin cannot be approved. The legal and scientific rationale for this conclusion is described below.

Any synthetic generic conjugated estrogens application based on Premarin as the reference listed drug is not to be approved until the active ingredients of Premarin have been sufficiently well defined to permit an ANDA applicant to establish that a synthetic generic form of Premarin has the same active ingredients as Premarin. In addition, I am requesting that the bioequivalence guidance for conjugated estrogens be examined to determine whether it should be revised in view of this position.

II. Legal Requirements for Approval of an ANDA

Under section 505(j)(2)(A)(ii)(II) of the Federal Food, Drug, and Cosmetic Act (the FD&C Act or the Act), 21 U.S.C. § 355(j)(2)(A)(ii)(II), an abbreviated new drug application (ANDA) that refers to a listed drug with more than one active ingredient must contain, among other things, "information to show that the active ingredients of the new drug are the same as those of the listed drug...." Section 505(j)(3)(C)(ii) of the Act, 21 U.S.C. § 355(j)(3)(C)(ii), requires that the Secretary shall approve

such an ANDA unless the Secretary finds, among other things, that "information submitted with the application is insufficient to show that the active ingredients are the same as the active ingredients of the listed drug...."

The implementing regulations provide that an ANDA not based on an approved suitability petition must provide information to show, among other things, that the active ingredients of the proposed and the reference listed drugs are the same (21 C.F.R. § 314.94 (a)(5)). FDA will refuse to approve an ANDA if "information submitted with the abbreviated new drug application is insufficient to show that the active ingredients are the same as the active ingredients of the reference listed drug" (21 C.F.R. § 314.127(a)(3)(ii)). The term "same as" means identical in active ingredient(s).[1] (21 C.F.R. § 314.92(a)(1))

The Agency has defined the term "active ingredient," as follows:

> any component that is intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body of man or other animals. (21 C.F.R. §§ 60.3(b)(2), 210.3(b)(7))

In the context of ANDA approvals, a generic product with the same active ingredients as the reference listed drug that is shown to be bioequivalent is approved without independent effectiveness data.[a] To meet the definition of an active ingredient in this context, a component must be intended to furnish sufficient pharmacological activity, or other direct effect, to have some therapeutic effect (i.e., to diagnose, cure, mitigate, treat, or prevent disease, or to affect the structure or function of the body). Thus, an active ingredient performs a drug's therapeutic functions. The definition of "pharmaceutical equivalents" in 21 C.F.R. § 320.1(c) is consistent with this definition of active ingredient in that it focuses on the therapeutic moiety:

> *Pharmaceutical equivalents* means drug products that contain identical amounts of the identical active drug ingredients, i.e., the same salt or ester of the same therapeutic moiety...that meet

[a] In enacting the Drug Price Competition and Patent Term Restoration Act of 1984, Congress intended that no safety or effectiveness data beyond that developed by the innovator company be needed to support approval of the generic product. (See H.R. Rep. No. 857 (Part I), 98th Cong. 2d Sess. 14, 16-17 (1984)). The interpretation of the active ingredient definition in this momorandum is intended solely as applied to ANDA approval.

identical compendial or other applicable standards of identity, strength, quality, and purity, disintegration times and/or dissolution rates.

Consequently, not all components that "furnish pharmacological activity or other direct effect" meet the definition of an active ingredient. A component may be considered an active ingredient only if it provides a clinically meaningful contribution to the therapeutic effect of the drug. A subjective intent for a component to have such effect will not suffice in the absence of objective evidence of a clinically meaningful contribution. (See 21 C.F.R. § 201.128; intended use refers to objective intent.)

In most cases, it will be clear what components of a drug make clinically meaningful contributions to the drug's therapeutic effects and, therefore, are the drug's active ingredients. However, where the Agency has determined there is sufficient evidence that a component in the reference listed drug may make a clinically meaningful contribution to the therapeutic effect, FDA cannot approve a synthetic generic drug that does not include such component until it has been determined whether the component makes such a contribution.

III. Regulatory History of Conjugated Estrogens

FDA first permitted a new drug application for Premarin (conjugated estrogens tablets made from pregnant mare's urine) to become effective in 1942 under the new drug provisions of the 1938 FD&C Act, Pub. L. 75-717, 52 Stat. 1040, based on chemistry, manufacturing, and controls information acceptable at that time and a showing, from reports of clinical investigations, that the drug product was safe for its intended use in the treatment of menopausal symptoms and related conditions. The product was known at that time to contain estrone and equilin, and it was known that additional estrogens were present in smaller amounts. The tablet strengths and estrogenic potencies of Premarin tablets were controlled using a colorimetric assay and a rat bioassay, respectively, with estrone as the reference standard. Thus, the 0.625 mg Premarin tablet was assigned this value because it contained estrogenic potency that, in the rat model, was equivalent to 0.625 mg of sodium estrone sulfate.

In 1970, the United States Pharmacopeia (USP) published monographs for conjugated estrogens and conjugated estrogens tablets, establishing the first compendial standards for these products.[2] The USP described conjugated estrogens as containing

sodium estrone sulfate and sodium equilin sulfate.[b] This description appears to have been based on the known quantity, in Premarin, of each of the two ingredients as well as their demonstrated clinical estrogenic effects.[3,4,5] The two compounds were known to be the most abundant estrogens in Premarin. Clinical data showing estrone to be an active estrogen were available, and small-scale clinical studies of sodium equilin sulfate indicated that it was a more potent estrogen than estrone.[6] Limited data from a study completed in 1963 and published in 1971 suggested that sodium 17"-dihydroequilin sulfate, the third most abundant estrogen, had little clinical activity.[7]

With the publication of the monographs in 1970, the rat potency test was eliminated and replaced by a chemical assay for the two active ingredients. However, the traditional strength assignment was maintained, even though the tablets contained fewer milligrams of sodium estrone sulfate and sodium equilin sulfate than the milligram dose stated on the label.

In 1972, FDA published an assessment of the effectiveness of Premarin.[8] Drugs such as Premarin that were approved prior to 1962 were required to demonstrate safety but not effectiveness at the time of approval. In 1962, enactment of the Harris-Kefauver amendments to the FD&C Act created a requirement for a demonstration of the effectiveness of new drugs including new drugs approved between 1938 and 1962 (Pub. L. 87-781, 76 Stat. 780). FDA contracted with the National Academy of Sciences/National Research Council to carry out the Drug Efficacy Study to assess the evidence of effectiveness available for new drugs approved prior to 1962. FDA then implemented the results in an effort known as DESI (Drug Efficacy Study Implementation). The 1972 *Federal Register* notice announced FDA's conclusion that a number of estrogen products, including Premarin, had been shown to be effective for menopausal symptoms (and several other conditions) based on the DESI Panel recommendations and other available evidence. FDA also found that the listed estrogen products were "probably effective" for prevention of

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[b]In the preamble to the final rule implementing Title I of the Drug Price Competition and Patent Term Restoration Act of 1984, FDA stated that, although in most cases the Agency will consider an active ingredient to be the same as that of the reference listed drug if it meets the standards of identity described in the USP, "in some cases, FDA may prescribe additional standards that are material to an ingredient's sameness." (See *Federal Register*, Vol. 57, p. 17950, 17959, April 28, 1992.) See also 21 C.F.R. § 320.1(c), which states that an identical active drug ingredient may meet "identical compendial **or other applicable standards"** (emphasis added). FDA applies current scientific knowledge in making its regulatory decisions, even if that knowledge has not yet been incorporated into the USP monograph.

osteoporosis. For indications found to be “probably effective,” FDA required sponsors to either submit substantial evidence of effectiveness or remove the indication from the product labeling within a certain period of time.

In 1978, Ayerst Laboratories proposed that conjugated estrogens be required to contain seven estrogenic components. Ayerst subsequently modified this proposal to request only that 17"-dihydroequilin be added to the existing USP monograph.[9] In 1982, FDA and USP convened a public meeting to discuss Ayerst Laboratories’ proposal that the monograph for conjugated estrogens include 17"-dihydroequilin.[10] FDA stated at that time that the composition of conjugated estrogens should be determined by estrogenic potency and that the proposed compound had low potency and likely did not contribute to the clinical effect. USP determined that 17"-dihydroequilin should not be added to the monograph as an active ingredient.

In 1980, FDA published the first version of the document now known as the *Approved Drug Products with Therapeutic Equivalence Determinations*, also known as the “Orange Book.”[11] This document lists the FDA assignment of therapeutic equivalence among duplicate drug products based on available data pertaining to their pharmaceutical equivalence and bioequivalence. Existing conjugated estrogens tablet products were classified as “BS,” i.e., not considered therapeutically equivalent, because of concern that the USP monograph specifications for estrone sulfate and equilin sulfate were inadequate to ensure that products meeting the monograph standard would necessarily produce equivalent therapeutic effects in patients.[12] The “BS” code is used by FDA to indicate that drug products are not considered therapeutic equivalents due to deficient drug standards.

In 1986, FDA announced in the *Federal Register* that a 0.625 mg dose of Premarin daily was found to be effective for prevention of osteoporosis in postmenopausal women.[13] Two dose response studies evaluating the effect of Premarin on bone mineral density had been published in the literature.[14,15]

In 1986, while developing an appropriate *in vitro* dissolution test standard for conjugated estrogens bioequivalence testing, FDA discovered that Premarin tablets were a modified release dosage form.[16] This unexpected characteristic of the Premarin formulation meant that generic copies were unlikely to be bioequivalent unless they also had similar modified release characteristics. Because of this discovery, FDA changed the “Orange Book” code for generic conjugated estrogens tablets from “BS” to “BP.”[17] The code “BP” means that generic products so

labeled are not considered therapeutically equivalent due to a potential bioequivalence problem. FDA then began to require that generic conjugated estrogens products demonstrate bioequivalence through *in vivo* human subject bioequivalence testing.[18] Because bioequivalence testing is ordinarily performed on the active ingredients of a product, the question of the active ingredients of Premarin again was raised.

In 1989, FDA's Fertility and Maternal Health Drugs Advisory Committee considered the question of the active ingredients in Premarin.[19] The Committee agreed that sodium estrone sulfate and sodium equilin sulfate are active ingredients, but could not reach a consensus on whether or not other estrogens in Premarin were active ingredients.[20] In 1990, an Ad Hoc Subcommittee of the Fertility and Maternal Health Drugs Advisory Committee met to consider Premarin bioequivalence issues.[21] Again, the group agreed that the two named active ingredients were correctly designated, but could not reach a consensus on whether additional components should be regarded as active ingredients.[22]

In 1990, FDA published a proposal to withdraw approval of the "BP" coded generic conjugated estrogens formulations for which therapeutic equivalence could not be ensured.[23] The proposal included withdrawing all generic conjugated estrogens marketed at that time. The Agency withdrew approval for these products in 1991, and there are currently no approved generic conjugated estrogens tablets on the U.S. market.[24,25]

In February 1991, FDA's Generic Drugs Advisory Committee met to consider issues of pharmaceutical equivalence and bioequivalence for conjugated estrogens.[26] FDA proposed to the committee that three of the additional estrogens in Premarin be recommended for inclusion as "concomitant components" in the USP monograph for conjugated estrogens.[27,28] These particular "concomitant components" would be required to be in the product, but would not be considered active ingredients and, thus, would not need to be included in bioequivalence testing.[29] The Generic Drugs Advisory Committee endorsed this proposal.[30] Subsequently, the USP monographs on conjugated estrogens were amended to include the three additional "concomitant components."[31]

On November 30, 1994, Wyeth-Ayerst submitted a citizen petition requesting, among other things, that FDA not approve any generic conjugated estrogens products that do not contain the compound sodium)8,9-dehydroestrone sulfate (DHES).[32] Wyeth-Ayerst also submitted a petition for a stay of action requesting that FDA stay any decision to "receive" an ANDA for a conjugated estrogens product that does not contain DHES and stay any approval of such

an application until FDA responds to the petition.[33]

Because of the complex scientific issues associated with determining the active ingredients of conjugated estrogens, in the summer of 1995, CDER formed an Ad Hoc Conjugated Estrogens Working Group to consider these issues. That group of CDER staff examined available data related to the composition of conjugated estrogens and prepared a background document for the Fertility and Maternal Health Drugs Advisory Committee.

On July 27-28, 1995, FDA's Fertility and Maternal Health Drugs Advisory Committee, with representation from FDA's Generic Drugs Advisory Committee and FDA's Endocrinologic and Metabolic Drugs Advisory Committee, heard presentations and discussions on the composition of conjugated estrogens.[34] At the end of the deliberations, in answer to questions regarding what additional components, if any, beyond the two recognized active ingredients contribute to the clinical safety and effectiveness of Premarin, the Committee voted unanimously in favor of the following statement:

> The Committee feels that insufficient data were presented to determine **whether or not** any individual component of Premarin or any combination of components in Premarin other than estrone sulfate and equilin sulfate must be present in order for Premarin to achieve its established levels of efficacy and safety [emphasis added].[35]

On November 1, 1996, FDA completed a "Preliminary Analysis of Scientific Data on the Composition of Conjugated Estrogens."[36]

On May 1, 1997, the Ad Hoc Conjugated Estrogens Working Group completed its final report providing a scientific background for the Center's decision regarding the composition of conjugated estrogens.[37]

The regulatory history of conjugated estrogens reflects the complexity of the scientific issues involved. FDA's positions on these issues have evolved over time as new information has become available. As with any such complicated scientific issue, differences in scientific opinion arose and continue to exist concerning how available data are to be interpreted and applied in the regulatory context. These differing views were considered in reaching the CDER position described in this memorandum. Three of these views were recently documented in memoranda to the Director, CDER, and are representative of the spectrum of views expressed during the Center discussions of these issues.[38,39,40]

IV. Characterization of Premarin

A. FDA's Historical Position On The Active Ingredients Of Premarin

Although FDA's Scientific Advisory Committees were unable to provide definitive advice on this issue, FDA continued to support the position taken in the 1970 USP monograph[41] that the ingredients sodium estrone sulfate and sodium equilin sulfate are the sole active ingredients in Premarin. The reasons for this position were as follows:[42]

1. Until recently, the scientific belief had been that all estrogens were similar in their pharmacologic actions on the body, i.e., "an estrogen is an estrogen." Therefore, the pharmacologic activity of an estrogen preparation could be described in terms of its total estrogenic potency. It was believed that the effects of different estrogens in a mixture were additive and that the identity of the particular estrogen contributing the estrogenic potency was not crucial. Epidemiologic data did not reveal safety or effectiveness differences among various estrogen preparations used for hormone replacement therapy.

   As a result, Premarin has historically been defined in terms of total estrogenic potency rather than the sum of the potencies of various components. In 1970, when the first USP monograph was published, little information was available on the effects of estrogens on bone, and the estimates of estrogenic potency of Premarin components were derived from clinical studies of menopausal symptoms. Much of Premarin's estrogenic potency for menopausal symptoms can be attributed to the effects of estrone and equilin.

2. Available data on the detailed composition of Premarin and the pharmacologic activity of its components were limited. Much of the available data indicated that many compounds found in Premarin were present in small amounts and had weak estrogenic activity.

3. Based on the results of early studies, including studies of Premarin, the effects of estrogen on bone mineral density appeared to have a very steep dose-response relationship, and the 0.625 mg dose of Premarin appeared to be near the top of the dose response curve. Therefore, small differences in the

estrogenic potency of conjugated estrogens preparations, resulting from omission of components from generic copies, would not be clinically meaningful.

4. In addition, the monograph ranges for the content of sodium estrone sulfate and sodium equilin sulfate in conjugated estrogens are wide.[43] Therefore, it was believed that minor differences in estrogen content between synthetic generic products and Premarin due to the absence in the generic copies of several minor Premarin constituents could not make a clinically meaningful difference. [Note: the percent coefficient of variation of sodium estrone sulfate is 1.98, and of sodium equilin sulfate is 3.01, based on percent estrogen composition in 500 batches of Premarin Tablets.[44]]

B. The Center's Current Position On Premarin's Active Ingredients

For the reasons described below, the Center's current position is that Premarin is not sufficiently characterized at this time to determine all of its active ingredients.

1. Emerging scientific evidence demonstrates that all estrogens do not exert their effects in a uniform manner with respect to different target tissues. These differential effects may be due to variable pharmacokinetics,[c] tissue metabolism, tissue-specific receptor factors, or additional reasons.[45,46,47,48,49,50] For example, clinical studies have shown that the potency of equilin sulfate relative to estrone sulfate varies depending on the pharmacodynamic[d] effect being studied.[51,7] A dose of equilin sulfate that is equipotent to estrone sulfate using one parameter may be more or less potent when evaluated using a different measure. For this reason, the active ingredients of Premarin cannot be defined solely in terms of overall estrogenic potency in any single system, but must be defined based on their contributions to particular

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[c]Pharmacokinetics can be defined as drug absorption, excretion, metabolism, or distribution.

[d]Pharmacodynamics can be defined as a pharmacologic or clinical response to a given concentration [of a drug] in blood or other tissue (58 FR 39409, July 22, 1993).

estrogenic effects.

Put simply, the new scientific evidence shows that one estrogen can be more active than another in a specific tissue or organ, such as breast, uterus, or bone. The most striking example of this is the synthetic estrogen analog tamoxifen, which blocks estrogen actions in breast tissue, but has estrogen-like activity on bone. These new findings have stimulated extensive research into new pharmaceuticals that could have selective actions on specific tissues and thus might provide beneficial hormone replacement therapy without some of the undesirable side effects, or could be useful in the treatment of cancer or other conditions.

2. Compositional analysis of Premarin using modern analytical techniques demonstrates that it consists of a mixture of a substantial number of compounds with potential pharmacologic activity. In fact, the steroidal content of Premarin has not been completely defined.[52] Undoubtedly, many of the compounds present in Premarin do not provide a clinically meaningful contribution to the therapeutic effects of the drug and are best thought of as impurities. However, the clinical tests, on which the findings of the safety and efficacy of Premarin were based, were performed on the entire mixture, not on individual components. A basic understanding of the chemical composition of Premarin must be achieved as a first step in adequately characterizing the product, unless a complete understanding of which components provide a meaningful clinical contribution to the effects of the product is achieved by clinical trials alone.

3. Clinical studies have revealed that the assigned potencies of Premarin tablets, which were based on the rat bioassay, do not correctly reflect the tablets' relative potencies in human studies.[50,51,7,53] For example, clinical studies have shown that Premarin is between 1.4 and 2.5 times more potent than estrone sulfate for suppression of FSH and menopausal symptoms in postmenopausal women.[50,7] Because the human studies evaluating the relative potency of Premarin have been small, a precise estimate of the estrogenic potency of Premarin relative to estrone sulfate has not been determined. Because the relative potencies of Premarin, estrone sulfate, and equilin sulfate are not clearly established, it is not possible to tell how much of the effect of Premarin can be accounted for by

the effects of equilin sulfate and estrone sulfate. Measuring these effects is further complicated by the fact that the importance or contribution of each ingredient may depend on the tissue that is being tested, e.g., bone, breast, pituitary, or uterus.

4. New clinical studies have clearly demonstrated that there is a dose-response relationship between estrogen administration and bone mineral density in postmenopausal women.[54,55] It follows that ensuring an equivalent estrogenic potency is important in the approval of generic copies of estrogen products intended for prevention of osteoporosis. In other words, it is important for the osteoporosis indication that synthetic generic conjugated estrogens based on Premarin have estrogenic strength that is identical to the Premarin tablet.

5. The recent findings with regard to)8,9-dehydroestrone sulfate (DHES) illustrate a number of the above points. This compound was first detected in Premarin in 1975.[56,57] DHES represents only a small percentage of the estrogenic compounds present in the product: 4.4% of the “label claim” (i.e., 4.4% of 0.625 mg or approximately 0.0275 mg of DHES per 0.625 mg tablet). [Note: Premarin also contains a small amount of the DHES metabolite sodium 17$-)8,9-dehydroestradiol sulfate.[58] This metabolite comprises approximately 0.003 mg per 0.625 mg tablet. Therefore, the total DHES plus sodium 17$-)8,9-dehydroestradiol sulfate content of a 0.625 mg tablet is about 0.03 mg or approximately 5% of label claim.] Until recently little has been known about DHES or sodium 17$-)8,9-dehydroestradiol sulfate.

   Pharmacokinetic studies submitted by Wyeth-Ayerst demonstrate that, after single or repeated oral dosing of Premarin in women, the plasma concentration or AUC’s of the (conjugated plus unconjugated) 17$-)8,9-dehydroestradiol metabolite of DHES is the same order of magnitude as the concentration of the 17$-diol metabolites of the active ingredients estrone and equilin.[59,60,61] The 17-$)8,9-estradiol concentration is approximately 34% of the combined concentrations of the 17$-diol metabolites of estrone and equilin, or 26% of the 17$-diol metabolites from the three estrogens. The finding that a low-level (5%) component of the tablet would generate a significant concentration of a

potentially active metabolite was completely unexpected and illustrates the longstanding inadequate characterization of Premarin. These pharmacokinetic data do not themselves prove that the DHES in Premarin makes a clinically meaningful contribution to the therapeutic effect of Premarin. However, preliminary clinical studies indicate that the potency of DHES may be similar to that of equilin. (See detailed discussion below.)

6. Based on this new scientific information, the Center concludes that Premarin is not adequately characterized and that, therefore, at this time, its active ingredients cannot be fully determined. Additional information on both composition and relative potencies of components will be necessary to adequately characterize this product. This conclusion is in agreement with the findings of FDA's Fertility and Maternal Health Advisory Committee at its July 27-28, 1995, meeting on this subject.[34]

C. Unresolved Issues Concerning the Current Characterization of Premarin

Products such as Premarin, that are derived from natural source material, frequently are not characterized as completely as synthetic products at the time of marketing. For the purposes of this memorandum, the term "adequate characterization" is intended to mean an amount of scientific information on a product that is sufficient to determine what constituents in the product are responsible for making clinically meaningful contibutions to its therapeutic effects. In other words, it is possible to define the active ingredients of a product that is adequately characterized.

There are at least two possible ways to characterize a product. The most straightforward method includes, first, chemical analysis to determine what components are present at significant levels in the product. The interpretation of "significant levels" cannot be exact and would depend on the specific product; however, it is desirable that components present at the 0.1% level or greater be identified and quantified. Once the components of the product are identified, the next step in characterization would be to determine which of them have potential human pharmacologic activity. Such a determination may be based on the following: the quantitative amount in the product, structure-function relationships, *in vitro* tests, animal

studies, human studies, or a combination of these. Finally, for components that may contribute to the therapeutic effect based on potential pharmacologic activity, a study could be conducted comparing the effects of each component alone, and in combination with additional components, to the effects of the entire product, to demonstrate that the “candidate” components achieved all of the therapeutic effects of the product.

Alternatively, in cases where there is some confidence that the “candidate” active ingredients have all been identified, even though the product is not fully chemically characterized, a head-to-head comparative dose-response clinical trial comparing the effects of the combined “candidate” active ingredients against the original product, could, if carried out carefully, demonstrate that the combination contributed all the clinically meaningful therapeutic effects of the original product. This approach might not clearly identify which of the “candidates” were actually active, but could ensure that the combination tested included all of the active ingredients in the product.

The following sections discuss the available scientific evidence on the characterization of Premarin.

1. Composition

At least ten estrogenic compounds have been identified and quantified in Premarin. The composition data for the ten estrogenic compounds cited in the Conjugated Estrogens, USP monograph, and listed in Table 1, were generated by the Center's Division of Drug Analysis from an analysis of two batches of Premarin 0.625 mg tablets.[62] These results agree generally with other data available to the Center.

Table 1

Sodium Estrogen Sulfate	Mg/Tablet
Estrone	0.370
Equilin	0.168
17"-Dihydroequilin	0.102
17"-Estradiol	0.027
17$-Dihydroequilin	0.011
l7"-Dihydroequilenin	0.011
17$-Dihydroequilenin	0.021

Equilenin	0.015
l7$-Estradiol	0.005
)8,9-dehydroestrone	0.026

Additional information on the component DHES and its metabolite are discussed later in this section (IV.C.4). Additionally, the fact that Premarin contains progestational agents (composition unspecified) has been disclosed by Wyeth-Ayerst.[63] It is known that Premarin also contains additional steroidal compounds.[52] However, precise data on Premarin's composition are currently very limited.[64,65,66,67]

Detailed analytical information on Premarin's composition is the necessary basis for adequate characterization of the product. Obtaining this information is feasible. The constituents of Premarin are small molecules that can be fully characterized by analytical chemistry, unlike the macromolecular constituents of most biological products, which are difficult to fully characterize due to biologic variability. It is desirable that the components present in Premarin at or above 0.1% be characterized and their biological activities determined.[68]

It has been argued that DHES cannot be considered an active ingredient of Premarin because its presence in and percent composition of the formulation are not specifically controlled during the manufacturing process.[69] Wyeth-Ayerst has submitted data demonstrating that DHES is present at about 4.4% of label claim with a range of 4.0 to 5% (based on ten lots of 0.625 mg Premarin tablets).[70] It is desirable that any active ingredients, once identified, be controlled during the manufacturing process.

2. Pharmacokinetics

Pharmacokinetic data on Premarin components are presented in the FDA report entitled *A Pharmacokinetic Analysis of Conjugated Estrogens Including)8,9 Dehydroestrone and 17$-)8,9 Dehydroestradiol*, dated October 25, 1996 (OCPB Report),[71] and its addendum dated February 12, 1997 (Addendum),[72] and also in information submitted to the docket of the Wyeth-Ayerst citizen petition by Wyeth-Ayerst.[59,60] The OCPB Report

details plasma concentrations of estrone sulfate, equilin sulfate, DHES, and their metabolites, as well as concentrations of 17"-dihydroequilin, after ingestion of various doses of Premarin.[72] Additional pharmacokinetic data on Premarin components and metabolites, presented in Addendum 2, dated March 31, 1997, to the OCPB Report,[73] and also in information submitted to the docket by Wyeth-Ayerst on March 11, 1997,[61] confirm the original finding discussed in the OCPB Report.

Table 2 is derived from pharmacokinetic data submitted by Wyeth-Ayerst based on seven-day dosing of women with two 0.625 mg tablets daily.[61] The steady-state AUC data are calculated from day seven plasma sampling. Table 2 summarizes the relationships among oral dose, total ketone, and total diol for three estrogens.

Table 2 - Results of Pharmacokinetic Studies

Estrogen	Estrone	Equilin	)8,9-DHE
Measured dose or AUC			
mg per 2X 0.625mg tab	0.740	0.336	0.052
Total plasma ketone (ng•hr/mL)	94.200	43.145	13.610
Uncon.plasma ketone (ng•hr/mL)	4.083	1.201	0.072
Total plasma 17$diol (ng•hr/mL)	8.565	10.623	6.624
Uncon.plasma 17$diol (ng•hr/mL)	0.659	1.060	0.331

The pharmacokinetics of Premarin components are complex, as revealed in these data. Estrone, equilin,)8,9-dehydroestrone, their active 17$-reduced metabolites, and other estrogenic components of Premarin circulate in the plasma both as the conjugated (primarily sulfate ester) and unconjugated derivatives and with various degrees of protein binding, as discussed in the OCPB Report. There is interconversion between the ketone and 17$-reduced forms of each estrogen and among the conjugated and unconjugated derivatives. The degree of protein binding of each derivative may be important to its clinical activity.

Put simply, this information shows that there is not a one-to-one relationship between the amount of each estrogen in the tablet and the amount of active forms (derivatives) of that estrogen in the blood. Each of the three estrogens evaluated in this clinical trial distributes differently into its derivatives in the body. This means that each of the three estrogens might cause different effects simply as a result of these distributional differences.

The actual magnitude of the contribution of each derivative of any component estrogen to the overall estrogenicity of Premarin is not well understood. As just stated, the pharmacokinetic data show that the ratios of the concentrations of the different derivatives are distributed differently for those estrogens that have been studied: estrone, equilin, and DHE. If there are tissue-specific effects of derivatives, then the size of a derivative's contribution could vary depending on the tissue tested. The available data suggest that these tissue-specific differences exist. For example, *in vitro* potency data for estrone and 17$-estradiol were submitted by Wyeth-Ayerst.[74] When potency was tested by estrogen receptor binding, estrone was shown to be much less potent than estradiol (about 200 times less), as has been previously shown by receptor binding and cellular assays. In contrast, when potency testing was performed in a liver (Hep-G2) cell line using functional activation, estrone's potency appeared to be of the same order of magnitude as estradiol's potency. The experimenters were able to show that this increased potency of estrone resulted from its conversion to estradiol by the cells. Therefore, in tissues that have the capability to metabolize ketone forms to diols (e.g., estrone to estradiol), circulating ketone forms could make a large contribution to observed effects in that tissue. Similarly, conversion of conjugated (sulfated) forms of circulating estrogens to the unconjugated forms has been shown to occur in target tissues such as breast.[75] In these tissues, total estrogen concentrations (i.e., conjugated plus unconjugated) may be more important than in tissues that cannot convert the conjugated forms to the active, unconjugated forms.

One striking finding in the pharmacokinetic data is the differences in the proportions of the 17$-diol

concentrations resulting from the three estrogens (sodium estrone sulfate, sodium equilin sulfate, and DHES), compared to the ratios of the three estrogens in the tablet. It is known that the 17$-diol derivatives of equilin and estrone are potent estrogens. The pharmacokinetic data as a whole show that, after dosing with Premarin, the plasma concentration of unconjugated 17$-dihydroequilin is about twice (1.6 times) as high as the concentration of 17$-estradiol, even though there is only about half as much equilin as estrone in the tablet. The difference in the concentration of the active metabolite may account for the known greater clinical estrogenic potency of equilin. As discussed above, an unexpected finding from the pharmacokinetic data in the Missouri study, the most reliable data generated to date, was that the plasma concentration of unconjugated 17$-)8,9- dehydroestradiol is about half the concentration of unconjugated 17$-estradiol, even though there is more than ten times more estrone sulfate than DHES in Premarin. This may account for the high oral potency of DHES that has been found in the limited clinical studies performed with this compound.[76,77]

Put simply, these data show that a dose of DHES results in a much higher blood level of the active metabolite than would result from the same dose of estrone sulfate. This finding alone suggests, but does not prove, that a low dose of DHES could have a much larger than expected effect.

The above pharmacokinetic data provide a basis for beginning to understand the complex relationship between the composition of Premarin and its clinical effects. However, this understanding is still incomplete. The pharmacokinetics must be understood in the context of pharmacodynamic properties of the various components, including their clinical effects.

3. Clinical effects of Premarin

Premarin and certain Premarin components have been tested fairly extensively in animals, particularly rodents. Animal data, either *in vitro* or *in vivo,* have not proven to be quantitatively predictive of the effects found in women.[78] Therefore, animal tests, while useful in screening compounds for activity, cannot be used to definitively assign human clinical

effects. The most confident conclusions can be drawn from human clinical testing. The following summarizes what is known about the contribution of Premarin components to its overall activity from *in vitro* or *in vivo* human testing.

a. Pharmacodynamics of Premarin and Some of Its Components

The term "pharmacodynamics" refers to pharmacologic or clinical responses to a given concentration of a drug in blood or other tissue.[e] For example, raising or lowering blood pressure, causing dry mouth, or constricting the pupils are pharmacodynamic effects of various drugs. Pharmacodynamic effects can be beneficial, harmful, or neutral. The benefits of most drugs derive from their desired pharmacodynamic effects, while drug side effects often result from undesirable pharmacodynamic activity.

Premarin and its components, like other estrogens, affect a wide variety of human tissues, including pituitary, breast, uterus, bone, liver, and endothelium.[47] Some of these actions result in the beneficial effects of the drug, some cause side effects, and some (for example, cardiovascular or lipoprotein effects) have not been definitively evaluated. There are studies in the literature of effects of estrogen on each of these tissues, especially effects on the pituitary, uterus, and bone. This section discusses the pharmacodynamic effects of Premarin and its components other than the relief of menopausal symptoms and prevention of osteoporosis.

A dose-response relationship exists between estrogen treatment and FSH suppression.[79] Some pharmacodynamic data on suppression of FSH, including dose-response data, exist for equilin sulfate, estrone sulfate, and Premarin (see also menopausal symptoms, below).[6,7,50,80] In a study of suppression of urinary gonadotrophins, equilin was found to be about twice as potent as Premarin and five times more potent than estrone sulfate for this effect, while Premarin was 2.5 times more potent than estrone sulfate.[7] In studies of human serum FSH levels, Premarin has been found to be about 1.4-2.0 times as potent as estrone sulfate.[81,50] These

[e]See footnote c, supra.

studies are in relative agreement.

The published data on the effects of Premarin and its components on uterine or vaginal markers are limited. Beck and Friedrich found equilin sulfate to be 2-3 times more potent than Premarin for effects on vaginal epithelium and endometrium.[82] Varma et al found Premarin to be twice as potent as estrone sulfate for endometrial changes.[81] Geola et al evaluated the dose-response relationship between Premarin and vaginal cytologies and concluded that 1.25 mg Premarin daily was necessary for achieving full replacement levels for this parameter.[80] These studies are not adequate for drawing firm conclusions about the relative contributions of equilin and estrone to the effects of Premarin on uterine or vaginal markers.

A number of studies of Premarin or its components have evaluated pharmacodynamic markers of bone effects.[51,15,79,80,83] Jones et al estimated that Premarin was twice as potent as estrone sulfate for reduction of the urinary calcium/creatinine ratio. This ratio is a measure of bone resorption. Geola et al performed a dose-response study evaluating the effect of Premarin on the calcium/creatinine ratio, and found that 0.3 mg Premarin was the lowest dose to have a significant effect. Lobo et al found that Premarin was twice as potent as both estrone sulfate and equilin sulfate for reduction of the urinary calcium/creatinine ratio. The Lobo finding of a significant effect of 0.3 mg Premarin was not duplicated in a larger study by Lindsay et al.[15] Because of limitations in study designs and because the pharmacodynamic markers for bone are not sufficiently quantitative, no conclusions about comparative pharmacodynamic effects on bone of Premarin or its components can be drawn from these results.

Data on Premarin or Premarin component effects on lipoproteins and other plasma proteins, or other pharmacodynamic markers are quite limited.[50,51,53,49,84] Having information about these effects is important for several reasons. Stimulatory effects on liver proteins may affect drug safety. In addition, as discussed in the OCPB Report,[71] levels of circulating unconjugated estrogens may be affected by binding to plasma proteins, particularly sex hormone binding globulin (SHBG). Stimulation of SHBG could alter drug availability. Available data suggest that certain Premarin components differ in the ability to stimulate

SHBG.[50] Human pharmacodynamic data on DHES submitted by Wyeth-Ayerst demonstrated that 1.25 mg estrone sulfate had a much greater effect on SHBG levels than did 0.125 mg DHES;[85] however, this result requires confirmation.

Taken as a whole, the available pharmacologic data demonstrate that estrone sulfate (as the piperazine salt), equilin sulfate, and Premarin have different pharmacodynamic effects when potency on various tissues is evaluated.[50,51,7,53] For example, in a single study, Premarin was found to be 1.4 times more potent than piperazine estrone sulfate (expressed as the sodium rather than piperazine salt) for FSH suppression, a pituitary effect.[50] In contrast, Premarin was 3.5 times more potent than estrone sulfate for stimulation of angiotensinogen and 3.2 times more potent for stimulation of sex hormone binding globulin (SHBG). Presumably, this difference arises because other components of Premarin contribute to these effects in a manner different from estrone sulfate. It is not known if these differential pharmacodynamic effects are completely attributable to the presence of equilin sulfate.

In summary, the two Premarin components that have been carefully studied, equilin sulfate and estrone sulfate, differ from each other and from Premarin in phamacodynamic profile. It is not well understood which of the pharamcodynamic actions are desirable and which contribute to unwanted side effects. Adequate characterization of Premarin will require an understanding, based on scientific data, of those Premarin components that contribute to the pharmacodynamic effects of Premarin.

b. Clinical Effects of Premarin Components

i. Menopausal symptoms

A number of clinical studies evaluating Premarin and Premarin components for the treatment of menopausal symptoms have been performed.[79,80,82,86] Equilin sulfate has been found to be about three times more potent than Premarin for alleviating vasomotor symptoms.[82] The data submitted by Wyeth-Ayerst on DHES show that DHES is more potent than estrone sulfate for these effects, but the

data are not adequate to precisely assign a potency.[76] Without dose-response studies to determine the potency of DHES for menopausal symptoms relative to the potency of estrone sulfate and equilin sulfate, the contribution of DHES to the activity of Premarin in treating menopausal symptoms cannot be determined. Similarly, without a head-to-head comparison of the dose-related effects of Premarin, estrone sulfate, and equilin sulfate in the treatment of menopausal symptoms, the extent of contribution of the two components to the overall estrogenic potency of Premarin for this effect also cannot be accurately determined, although it is clear that both contribute.

ii. Osteoporosis prevention

Use of surrogate markers. The goal of preventive therapies for osteoporosis is the prevention of fractures and deformity. For estrogens, FDA accepts measurement of bone mineral density as an adequate surrogate for preventing these longer term clinical outcomes.[87] A number of other markers for evaluating pharmacodynamic effects on bone have been developed.[88] None of these other markers is sufficiently well understood or quantitative to permit its use as a surrogate for osteoporosis prevention effects. Therefore, in the absence of other validated surrogate markers, definitive data on bone effects must come from human trials evaluating bone mineral density, fractures, and/or deformity.

Use of blood 17$-estradiol levels as a surrogate marker. Comments submitted to the docket of Wyeth-Ayerst's citizen petition,[89] as well as statements in the scientific literature, assert that achievement of certain levels [e.g., 39 pg/ml (Palacios et al) or greater than 60 pg/ml (Reginster et al)] of serum 17$-estradiol is an adequate surrogate for preservation of bone mineral density because there is a strong correlation between the two both in clinical trials and in untreated perimenopausal women.[83,90]

The study by Palacios et al evaluated women who had undergone surgical menopause and who were randomized to percutaneous estradiol, conjugated

estrogens (source unspecified), or no therapy over two years. Untreated women lost a mean of 9% of spine bone mineral density over two years, whereas the estradiol treated group and the conjugated estrogens treated group gained 4.1% and 5.6% spinal bone mineral density respectively. Women treated with percutaneous estradiol were reported to have a mean serum estradiol level of about 80 pg/ml over the course of the study. The conjugated estrogens treated women had a mean serum estradiol level of about 40 pg/ml. It is not possible to conclude anything about a protective level of 17$-estradiol from the conjugated estrogens arm of this study since conjugated estrogens also contain, at a minimum, equilin and possibly other components that contribute to the effect on bone. The value of 80 pg/ml from the percutaneous estradiol arm is not inconsistent with the data reported by Reginster et al who found that circulating level of 17$-estradiol between 60-90 pg/ml correlated well with pharmacodynamic markers of beneficial bone effects. This correlation suggests, but does not prove, that estrogen replacement therapies achieving such levels of circulating estradiol may be effective in preventing bone loss.

FDA does not currently accept 17$-estradiol levels as an adequate surrogate for osteoporosis prevention in women. Trials of bone mineral density are required. In addition, the available data do not indicate that the potentially protective levels of 17$-estradiol are attained after admimistration of Premarin.

The Palacios study found that treatment with conjugated estrogens 0.625 mg resulted in a mean estradiol level of 40 pg/ml, which is below the 60 pg/ml minimum suggested by Reginster. However, the Librach and Nickel study submitted to the docket, as well as the Reginster study and other data reported in the literature, found that serum levels of 17$-estradiol above 60 pg/ml are achieved in women treated with Premarin or a Canadian generic copy of Premarin[89,91] In the Librach and Nickel study, women treated with Premarin achieved a 17$-estradiol level of 85.5 pg/ml while women treated with the Canadian product had mean serum levels of 94.9 pg/ml.

These differences appear to relate to problems with analytical methodology, possible due to cross-reactivity of radio-immunoassay reagents with other components in Premarin. When serum 17$-estradiol is measured by direct chemical means, the high 17$-estradiol levels are not found in women treated daily with 0.625 mg Premarin.[60,61] This latter finding is corroborated by data from a study of the effects of esterified estrogens (Estratab, USP) on bone mineral density, which was recently presented in abstract.[92] In this study, daily dosing with 0.625 mg of esterified estrogens, which contains approximately 0.518 mg sodium estrone sulfate[93] (0.625 mg Premarin contains about 0.370 mg sodium estrone sulfate) resulted in a mean plasma concentration of 17$-estradiol of 40 pg/ml. In addition, in this same study, daily administration of 0.3 mg esterified estrogens, which contain about 0.248 mg sodium estrone sulfate, resulted in a mean plasma concentration of 26 pg/ml of 17$-estradiol. These results are inconsistent with the serum level results presented by Librach and Nickel, but generally agree with Palacios' findings and with Wyeth-Ayerst's bioavailability data. Therefore, the available data on serum 17$-estradiol levels do not indicate that levels over 60 pg/ml are attained with the dose of Premarin recommended for the prevention of osteoporosis.

Clinical effects on bone. The clinical effects of Premarin on bone are well established. A number of clinical trials have confirmed the effects of Premarin in preserving and increasing bone mineral density in postmenopausal women.[14,15,94] Ettinger et al demonstrated in a nonrandomized trial that 0.3 mg Premarin, when administered with calcium supplementation, was adequate to prevent bone mineral loss in the spine and hip.[95] The recent PEPI trial demonstrated that the currently recommended 0.625 mg dose of Premarin resulted in an increase in bone mineral density in women treated for over two years, while untreated women lost bone.[96]

Estrone is approved as a single estrogen (marketed under the brand name Ogen by Upjohn, generic name estropipate), but as a different salt from the estrone in Premarin (the piperazine rather than

the sodium salt of estrone sulfate) for the treatment of menopausal symptoms and the prevention of osteoporosis. The recommended dose for osteoporosis is 0.75 mg of estropipate, which is equivalent to 0.625 mg sodium estrone sulfate. A dose-response study has shown that a dose equivalent to 0.300 mg estrone sulfate, combined with 1 gram daily calcium supplementation, is not effective in preserving bone mineral density.[97] In this study, 0.625 mg of estrone sulfate resulted in preservation of bone mineral density compared to baseline. There was no statistically significant difference in bone mineral density between patients dosed with 0.625 mg and those given 1.25 mg; however, only the 1.25 mg group had bone mineral densities statistically greater than the placebo group at two-year follow-up. Based on the data from this trial, the amount of estrone sulfate in Premarin (approximately 0.370 mg) is too small to account for all of Premarin's known effects on bone mineral density, so other estrogens present in the product must be contributing to this effect.

Additional information on the effects of equilin on bone has recently become available. On October 30, 1996, Duramed Pharmaceuticals submitted to the docket an abstract of a clinical study that had recently been presented at a scientific meeting.[89] The study provided new information germane to the clinical effects of Premarin on bone.[55] This study, sponsored by Solvay Pharmaceuticals, was a clinical trial of their product, Estratab (this trial was also discussed in the section on estradiol blood levels). Estratab is a generic esterified estrogens product. Esterified estrogens USP contain sodium estrone sulfate and sodium equilin sulfate in different amounts than are in Premarin[98] (based on presentations by Solvay, 0.300 mg of their esterified estrogens product contains approximately 0.248 mg estrone sulfate and 0.038 mg equilin sulfate).[93] The study was a two-year placebo controlled trial testing three doses of Estratab combined with calcium supplementation in postmenopausal women evaluating bone mineral density and side effects. According to the abstract, all three doses were effective at 12, 18, and 24 months in preserving

bone mineral density compared to placebo. The abstract reveals a dose response among the three Estratab doses tested. Also significant is the fact that the lowest dose tested, 0.3 mg Estratab, appeared to be effective in preserving bone mineral density when given continuously in conjunction with calcium supplementation. There are lower amounts of both estrone sulfate and equilin sulfate in this dose of Estratab than are required to be in the 0.625 mg tablet of generic conjugated estrogens according to the current conjugated estrogens USP monograph. Therefore, if the data in the abstract are correct, it could be concluded that a product containing the amounts of estrone sulfate and equilin sulfate required in the current monograph for conjugated estrogens USP would be effective in preserving bone mineral density when given continuously with supplemental calcium. Since the study by Harris, et al.[97] showed that 0.3 mg of estrone sulfate alone is not effective in preserving bone mineral density, then it is likely that there was a contribution from the equilin sulfate in the Solvay product, although firm conclusions cannot be drawn from cross-study comparisons. This information addresses to some extent one of the questions raised in FDA's *Preliminary Analysis of Scientific Data on the Composition of Conjugated Estrogens*,[36] that is, the fact that the contribution of equilin to preserving bone mineral density had not been demonstrated.

Despite this additional information, the question of what are the active ingredients in Premarin for the indication of maintaining bone is not completely resolved. The Solvay study demonstrated a dose response for bone mineral density. The lowest dose, 0.3 mg, was effective in preserving bone density. The two higher doses, 0.625 mg and 1.25 mg, of esterified estrogen actually increased bone density over the two-year period. This finding is consistent with other published data.[54,61] In the case of the Solvay study, it is not known whether, at the higher doses, more women responded with bone preservation than at lower doses, or whether women who would have responded to 0.3 mg simply had a larger response to the higher doses. In either case,

estrogenic potency has been shown to be important to the clinical effect on bone within this dose range. It has been estimated that a proportion of women taking the recommended dose of Premarin continue to lose bone mineral, even though mean values are sustained or improved.[99]

The finding that sodium equilin sulfate and sodium estrone sulfate, at the doses present in Estratab, preserve bone mineral density provides support for the proposition that equilin contributes to the bone preservation effects of Premarin. However, as discussed at the beginning of this memorandum, the requirement for approval of an ANDA is not that generic drugs have effects similar to the reference listed drug but, rather, that they have the same active ingredients. Only if the active ingredients are the same can generic copies be relied upon to have the same estrogenic potency and, therefore, the same effects on bone.

Limited data on the pharmacodynamic effects of DHES on bone have been submitted by Wyeth-Ayerst.[76,77] These data show that DHES has a pharmacodynamic effect on bone markers, but the data do not shed light on whether the DHES component of Premarin has a meaningful clinical effect on bone.

iii. Safety

There are safety concerns about all estrogen preparations currently approved for long-term administration for the prevention of osteoporosis. Long-term estrogen administration is associated with an increased incidence of endometrial cancer in women who have not undergone hysterectomy, and there is an ongoing controversy about the relationship of long-term estrogen replacement therapy to breast cancer.

No head-to-head studies have compared the long-term safety of various estrogen preparations when used chronically for the prevention of osteoporosis. The available epidemiologic evidence, summarized at the July 27-28, 1995, Advisory Committee meeting, does not definitively establish safety differences among various estrogens.[100] Thus, it is not known to what

extent, if any, differences in the types of estrogens used may affect safety.

There are no comparative safety trials of Premarin components available. There are few pharmacodynamic markers available with which to assess safety for effects such as cancer. Therefore, sufficient clinical data do not exist to fully characterize the contributions (either positive or negative) of various Premarin components to its clinical safety.

iv. Other pharmacologic effects.

There is currently intense interest in the role of estrogen replacement therapy (ERT) in the prevention of cardiovascular disease and possibly other age-related disorders in women.[101] No estrogen product is currently approved by FDA for such indications. If Premarin were to be found effective for prevention of cardiovascular disease, elucidating the effects of Premarin and its components on relevant pharmacodynamic parameters would be important in fully characterizing the product. There are clinical data suggesting that equine estrogens may have differential effects on parameters such as lipoprotein levels and lipid peroxidation;[51,84] however, these data are as yet very incomplete.

4. Inclusion of)8,9-dehydroestrone sulfate (DHES).

Many of the issues raised by Wyeth-Ayerst in its citizen petition submitted in November 1994, and addressed in numerous submissions to the docket of the citizen petition, pertain to the need to include DHES in generic copies of Premarin. Although this memorandum is not intended to be a response to the citizen petition and should not be construed as one, the scientific issues related to this compound are addressed below insofar as they relate to the approvability of generic copies of Premarin, which is the subject of this memorandum.

As discussed previously at the beginning of this section (IV.B.5.), DHES is a conjugated estrogens compound that comprises about 4.4% of the “label claim” of Premarin. It has been recognized as a constituent of Premarin for two decades.[57] However, little

scientific data have been available on its activity, and it has been treated as an impurity. Information submitted by Wyeth-Ayerst on the pharmacokinetics of DHES in Premarin reveal that its metabolite, 17$-)8,9-dehydroestradiol, is present in surprisingly large concentrations in the plasma, considering the composition of the tablet.[59,60] FDA analyses support this finding.[71] The 17$-)8,9-dehydroestradiol concentration is important because the diol form of estrogen is usually the most active in the human body. After taking Premarin, the concentration (or AUC) of unconjugated 17$-)8,9-dehydroestradiol in the plasma is between 50% and 125% (depending on what study results are used) of the concentration of unconjugated 17$-estradiol and is one third the concentration of unconjugated 17$-dihydroequilin.

The fact that a compound is present at high concentrations in the plasma does not necessarily mean that it is clinically important. The significance of the finding that 17$-)8,9-dehydroestrodiol is present in high concentrations depends on the potency of 17$-)8,9-dehydroestradiol compared to the potency of the other circulating estrogens. If it is assumed that the potency of the 17$-diol metabolites derived from estrone sulfate, equilin sulfate, and DHES have equal potency, then the contribution of DHES to the overall estrogenic activity of the 17$-diol metabolites of the three estrogens would be 16% (based on unconjugated diol AUCs) to 26% (based on total diol AUCs).[61] However, there are several ways to evaluate relative potency of estrogens. One method, testing in animal species, is useful for determining estrogenicity, but has not proven to be quantitatively predictive for humans (the original rat potency test for conjugated estrogens is a good example). This could be due to interspecies differences in metabolism, some of which have been confirmed.[102]

If animal testing is not adequately quantitative, *in vitro* studies using human cells or receptors may be performed, or human clinical tests may be carried out. Scientific data of both types assessing the relative potency of DHES have been submitted to the docket. Wyeth-Ayerst provided data on human estrogen receptor binding as well as functional activation data in HEP-2 cells.[103] In addition, Duramed Pharmaceuticals provided data on functional activation of Ishikawa cells, a

human uterine cell line.[104] The results of these studies are summarized in the OCPB Report of October 25, 1996,[71] Addendum 1 to that report dated February 12, 1997,[72] and Addendum 2 to that report dated March 31, 1997.[73] These OCPB Reports attempt to quantify the clinical estrogenic contribution to Premarin from equilin, estrone, DHES, and 17"-dihydroequilin based on the potencies derived from the various *in vitro* assays in combination with the pharmacokinetic data.

The OCPB Report estimates that, based on the *in vitro* potencies and the known pharmacokinetics, DHES and its metabolite contribute approximately 2.8-6.5% of the overall estrogenic potency of Premarin, depending on the assumptions used.[105]

Just as with the animal data, it is important to try to assess how reliably the *in vitro* data predict the actual clinical outcomes. A limitation of cellular assays is that only one tissue type is evaluated. The results of the OCPB analysis shows that widely differing estimates are arrived at depending on the system used.[106] This may be due to artifacts of the system (i.e., metabolism of estrone to estradiol, etc, in the Hep-G2 cells), true tissue differences, or other reasons. The best way to evaluate the *in vitro* potency assignments is to compare their results with known clinical outcomes. In this case, certain comparisons are possible because both estrone sulfate and equilin sulfate have been tested in women as single ingredients.[51,7] A number of clinical studies have shown that, for both FSH suppression and treatment of menopausal symptoms, equilin sulfate is roughly five times more potent than estrone sulfate when administered as a single ingredient. Comparison of this known clinical fact to the potency estimates in Tables 3 and 4 of OCPB Addendum 2 reveals that the Ishikawa cell potencies do not correctly predict the oral potency of equilin relative to estrone.[73] The Ishikawa cell data predict that oral equilin sulfate would be equipotent to or less potent than estrone sulfate. Of the other in vitro estimates, the estrogen receptor binding assay best predicts the known differences between equilin and estrone, predicting equilin sulfate to be between two to four times more potent than estrone sulfate depending on the assumptions used. Because of these widely differing estimates, it must be concluded that *in vitro* assays, even in human systems, cannot currently be relied upon

to provide precise predictions of relative clinical potencies.

The other information available on the relative potency of DHES comes from human studies. Wyeth-Ayerst submitted the results of two human studies to the docket.[76,77] These studies were small, unblinded, uncontrolled trials, and would not be of the type relied upon for determining safety or efficacy of a drug. In addition, they did not use a dosage form equivalent to that of Premarin, and thus their results cannot be directly extrapolated to Premarin. However, they are quite similar to the types of studies that were originally used to evaluate the role of estrone sulfate and equilin sulfate in Premarin and can be used to assess certain comparative pharmacodynamic parameters. In these trials, 0.125 mg of DHES was administered daily to postmenopausal women. This dose of DHES is about four times the amount in a 0.625 mg tablet of Premarin. In both studies, this dose of DHES caused approximately 15-26% suppression of FSH after two weeks of dosing. This is in the range of suppression resulting from 0.625 mg of estrone sulfate reported in the literature.[50] The study performed in Brazil included a comparison group given 1.25 mg estrone sulfate. This group achieved approximately a 40% reduction in FSH levels at two weeks. This effect is somewhat greater than has been previously reported.[50,81]

Based on these human data, the oral potency of DHES (for pituitary pharmacodynamic parameters) is (very roughly) five to six times that of estrone sulfate, or very similar to that of equilin sulfate and is about what would be predicted on pharmacokinetic grounds if the estrone and DHE derived diols were roughly equipotent. DHE, like equilin, is a B ring unsaturated estrogen. If DHES has the same oral potency as equilin and if the contributions of estrone sulfate, equilin sulfate, and DHES plus the small amount of 17$-)8,9-dehydroestradiol sulfate were to be considered, then DHES and its metabolite would contribute about 9% of the estrogenic potency from these three components, at least for pituitary parameters.

It can be seen from the above analysis that the high end of the estimate of the contribution of DHES to the estrogenic potency of Premarin from the *in vitro* assays

is similar to the estimate derived from clinical studies, i.e., about 9%, and both of the estimates are lower than the 16% to 26% estimate based on an assumption that each 17$-diol metabolite is equally potent. Unfortunately, all of the estimates have problems and uncertainties. A precise estimate of the potency of DHES relative to estrone sulfate is not available. In addition, none of the data provide insight into the contribution of these components to estrogenic potency with respect to bone. As discussed above, preliminary pharmacodynamic data indicate that DHES has an effect on bone markers. The available data demonstrate that DHES is a potent estrogen and may make a clinically meaningful contribution to the therapeutic effects of Premarin.

V. Conclusions

1. Under the Federal Food, Drug, and Cosmetic Act, for a generic drug product with Premarin as the reference listed drug to be approved, the generic drug must have the same active ingredients as Premarin. This requirement, paired with a showing of bioequivalence of the generic drug to the reference listed drug, is meant to ensure that the data developed by the innovator company to demonstrate the safety and effectiveness of the reference listed drug will support approval of the generic drug. Independent demonstration of safety and effectiveness is not required for approval of generic drugs. Approval of generic copies of Premarin manufactured from combined synthesized components will require data sufficient to demonstrate that such copies contain the same active ingredients as Premarin.

2. The reference listed drug Premarin is not adequately characterized at this time. In particular, the estrogenic potency of the product is not clearly defined relative to the estrogenic potency of its constituents. In addition, the contribution of the two most abundant estrogens, sodium equilin sulfate and sodium estrone sulfate, to the overall estrogenic potency is not well understood. Furthermore, the quantitative composition of Premarin with respect to potentially pharmacologically active components has not been defined. Without this information it is not possible to define the active ingredients of Premarin.

3. Investigations designed to produce the scientific data

needed to determine the active ingredients are feasible. Such information would allow a determination of which components of Premarin make a a clinically meaningful contribution to its overall effects. It is both feasible and desirable for the constituent active ingredients in Premarin to be characterized to this extent.

4. With regard to sodium)8,9-dehydroestrone sulfate (DHES), the available scientific evidence indicates that DHES is an active estrogen that contributes to the estrogenic potency of Premarin. The clinical significance of this contribution has not been determined. DHES must be included in generic copies of Premarin unless scientific data are presented that demonstrate that the estrogenic activity of DHES is not clinically meaningful.

5. Despite the fact that at this time Premarin is not adequately characterized, the Agency could approve generic copies of Premarin that originate from the same natural source material (pregnant mares' urine) before the active ingredients are defined, provided that detailed chemical composition of the product is known. This is because Premarin is manufactured and controlled using certain methods, and there could be confidence that generic copies using the same source materials and controlled in the same manner, based on the known composition of Premarin, would have the same level of assurance that the same active ingredients are in the generic product as are in Premarin.

6. In summary, the Center concludes that because the reference listed drug Premarin is not adequately characterized at this time, the active ingredients of Premarin cannot now be defined. Until the active ingredients are defined, a synthetic generic version of Premarin cannot be approved.

Janet Woodcock, M.D.

ENDNOTES

1. FDA, “Abbreviated New Drug Application Regulations; Proposed Rule,” *Federal Register*, Vol. 54, No.130, pp. 28872 (28880, 28881), July 10, 1989. States that to be “the same,” active ingredients must be “identical.”

2. *United States Pharmacopeia* 18, pp. 242-246, 1970.

3. Minutes of the meeting of the Committee on Conjugated Estrogens of the Pharmaceutical Contact Section, Washington, DC, October 23, 1962.

4. Minutes of the meeting of the Committee on Conjugated Estrogens of the Quality Control Section, Washington, DC, April 16, 1963.

5. Summary of Proceedings of the USP Conference on Conjugated Estrogens, February 27, 1968.

6. Howard RP, Keaty EC, Reifenstein EC, “Comparative effects of various estrogens on urinary gonadotropins (FSH) in oophorectomized women,” [Abstract] *J Clin Endocrinol Metabolism*, 16:966, 1956.

7. Howard RP, Keaty EC, “Evaluation of equilin 3-monosulfate and other estrogens,” *Arch Int Med*, 128:229-234, August 1971.

8. FDA, “Certain Estrogen-Containing Drugs for Oral or Parenteral Use,” *Federal Register* Vol. 37, No. 143, p. 14826-14828, July 25, 1972.

9. Minutes of the meeting on proposed USP monograph for conjugated estrogens, p. 4, November 4, 1982.

10. See note 9, pp. 1-4.

11. FDA, “Therapeutically Equivalent Drugs,” *Federal Register*, Vol. 44, No. 9, pp. 2932-2953, January 12, 1979. Announced that FDA intended to make available a list of approved drug products with therapeutic evaluations of products available from more than one manufacturer. Originally known as *Approved Prescription Drug Products with Therapeutic Equivalence Evaluations*, it is now called *Approved Drug Products with Therapeutic Equivalence Evaluations*, (the Orange Book).

12. 1980 Orange Book listing for conjugated estrogens, pp. 51-52.

13. FDA, “Oral Estrogens for Postmenopausal Osteoporosis; Drug Efficacy Study Implementation; Reevaluation,” *Federal Register*, Vol. 51, No. 70, p. 12568-12570, April 11, 1986.

14. Genant HK, Cann CE, Ettinger B, Gordan GS, “Quantitative computed tomography of vertebral spongiosa: a sensitive method for detecting early bone loss after oophorectomy,” *Ann Int Med,* 97:699-705, 1982.

15. Lindsay R, Hart DM, Clark DM, “The minimum effective dose of estrogen for prevention of postmenopausal bone loss,” *Obstet Gyn*, 63:759-763, 1984.

16. FDA, “Abbreviated New Drug Applications for Conjugated Estrogens; Proposal to Withdraw Approval; Opportunity for a Hearing,” *Federal Register*, Vol. 55, No. 30, pp. 5074, 5076-5078, February 13, 1990.

17. See note 16, p. 5076.

18. FDA, Center for Drug Evaluation and Research, Division of Bioequivalence, *Guidance for “In-Vivo Bioequivalence Study for Conjugated Estrogens Tablets*,” December 17, 1986.

19. Transcript, Vol II, and Summary Minutes of the meeting of FDA’s Fertility and Maternal Health Drugs Advisory Committee, January 5-6, 1989.

20. See note 19, Vol. II, pp. 177-193.

21. Transcript and Summary Minutes of the meeting of the Ad Hoc Subcommittee of FDA’s Fertility and Maternal Health Drugs Advisory Committee, Vols. I and II, May 3-4, 1990.

22. See note 21, Vol. II, pp. 117-135.

23. See note 16.

24. FDA, “Conjugated Estrogens Tablets; Withdrawal of Approval of 28 Abbreviated New Drug Applications,” *Federal Register*, Vol. 56, No. 57, p. 12376, March 25, 1991.

25. FDA, “Zenith Laboratories; Conjugated Estrogens Tablets; Withdrawal of Approval of Four Abbreviated New Drug Applications,” *Federal Register*, Vol. 56, No. 87, p. 20621, May 6, 1991.

26. Transcript of the meeting of FDA's Generic Drugs Advisory Committee, Vols. I and II, February 25-26, 1991.

27. See note 26, Vol. I, pp. 46-68.

28. Adams WP, presentation slide from the meeting of the FDA's Generic Drugs Advisory Committee, February 25, 1991.

29. See note 26, Vol. I, pp. 68-91.

30. See note 26, Vol. II, pp. 16-26.

31. USP, *Pharmacopeial Forum*, Vol. 17, No. 6, pp. 2-3, December 1991.

32. Wyeth-Ayerst submission to the docket 94P-0429 (CP 1), November 30, 1994.

33. Wyeth-Ayerst submission to the docket 94P-0430 (PSA 1), November 30, 1994.

34. Transcript and Summary Minutes of the meeting of FDA's Fertility and Maternal Health Drugs Advisory Committee, Vols. I and II, July 27-28, 1995.

35. See note 34, Summary Minutes, p. 5. See also note 34, Vol. II, pp. 296-297.

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